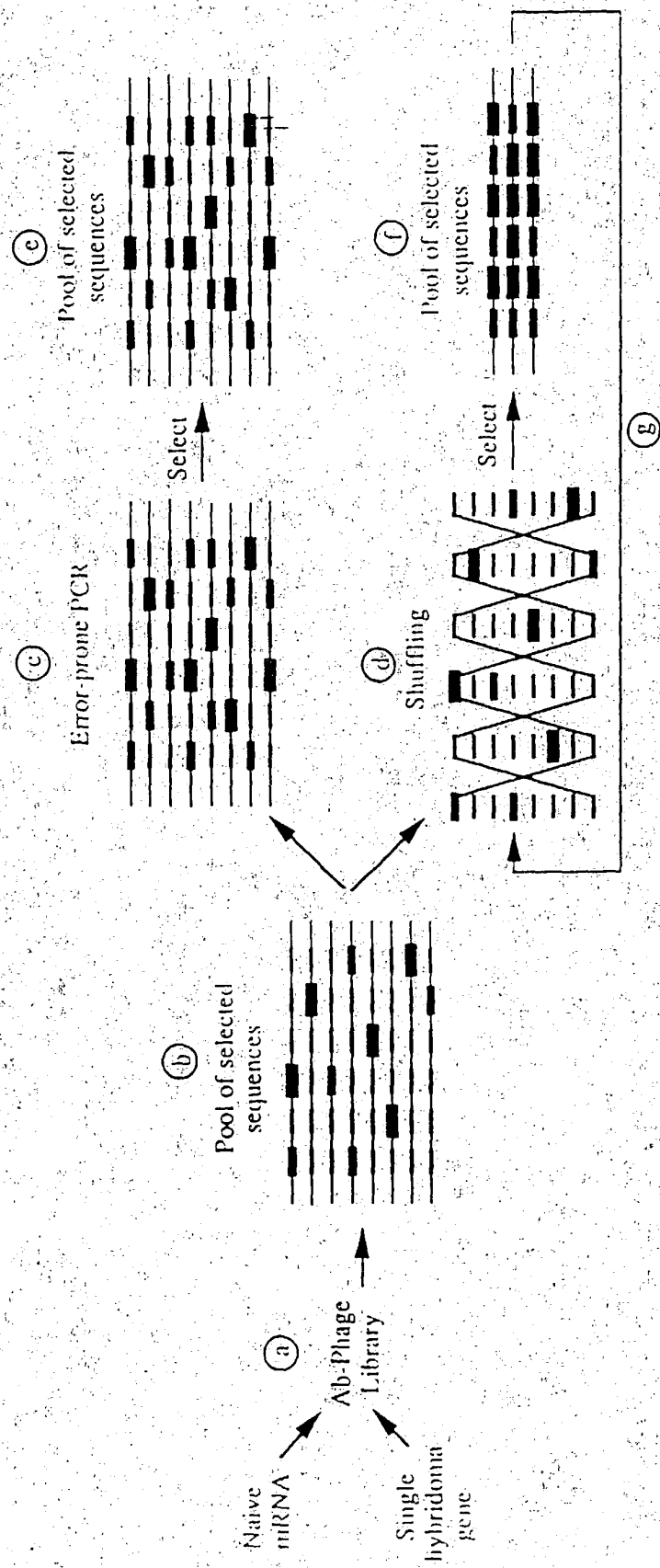


Fig. 1
TOP



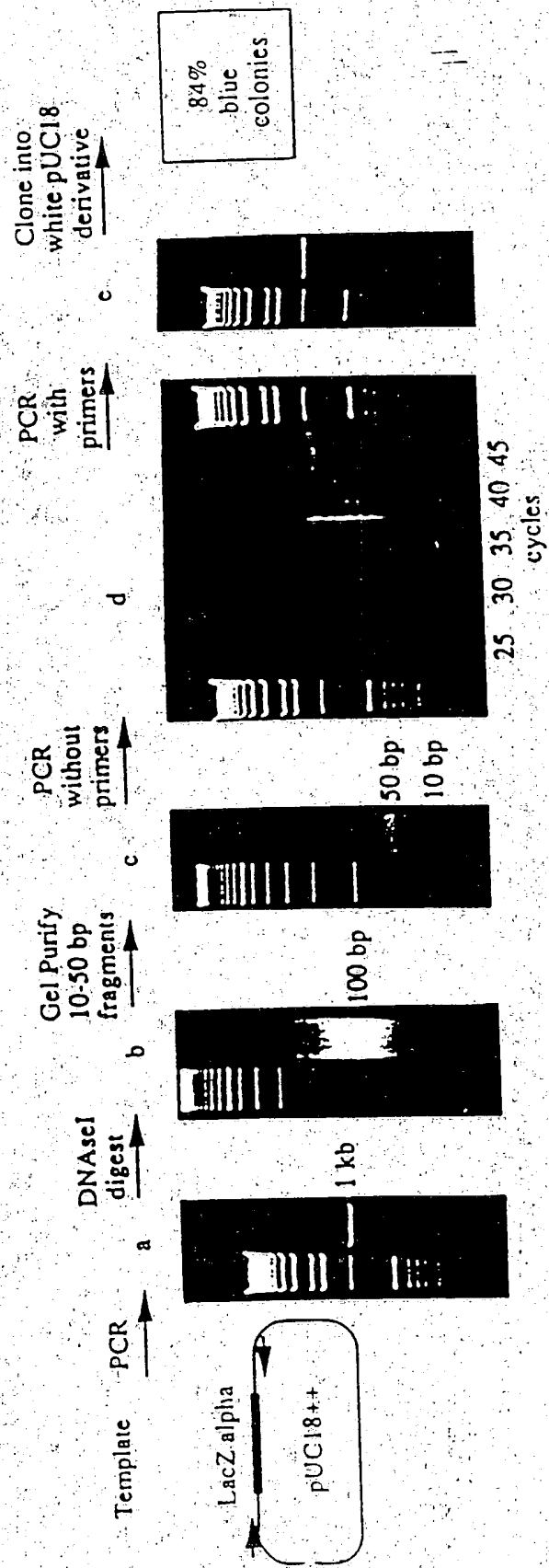


FIG. 2

Figure 3

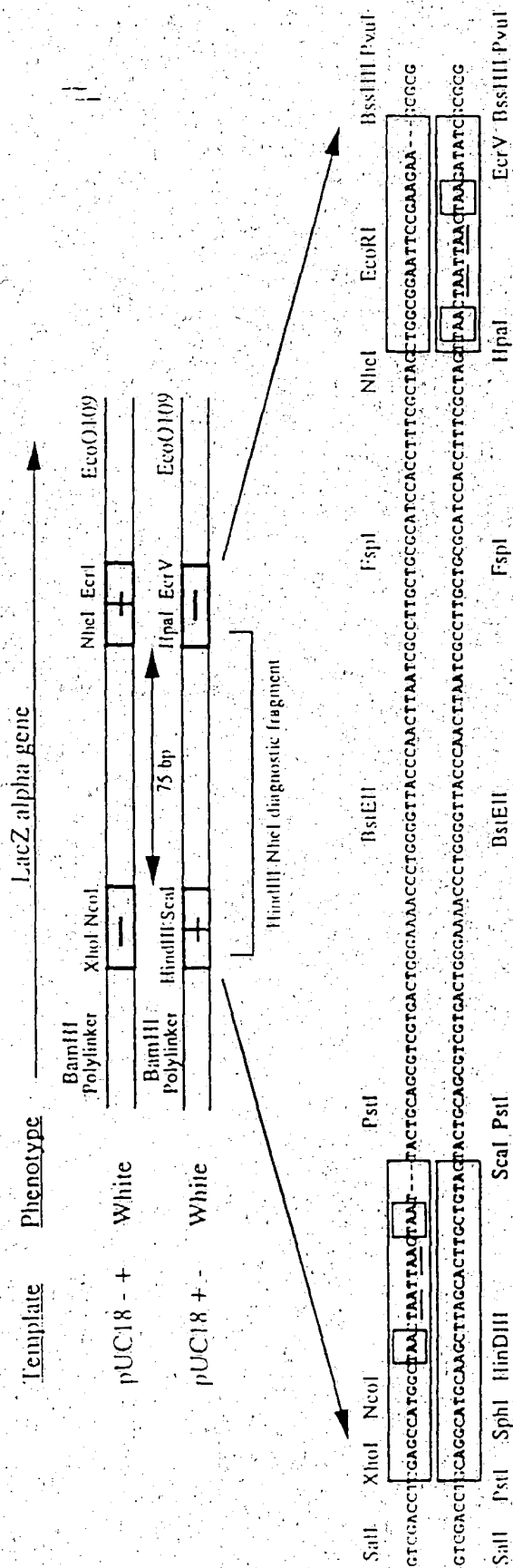
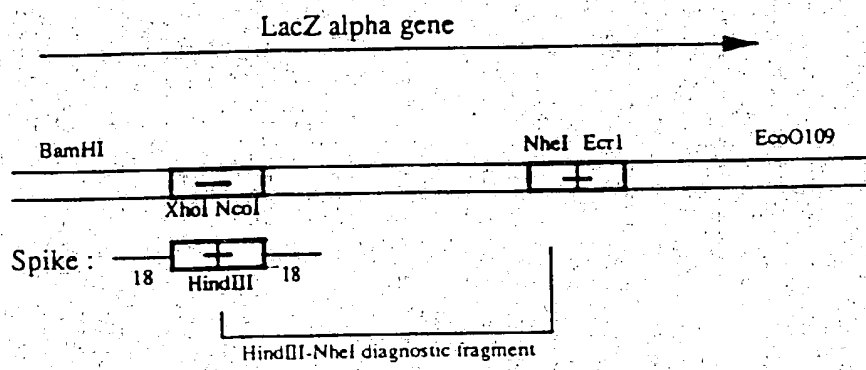
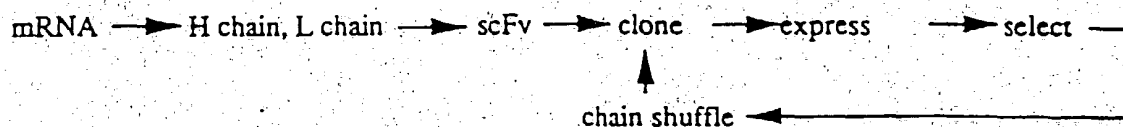


Figure 4

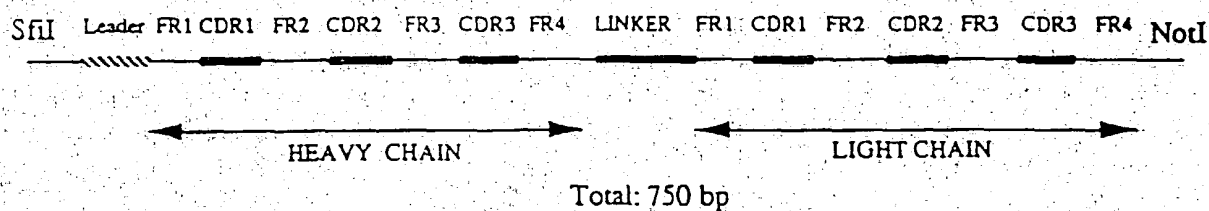


M CGGACGGGTCATCAGCGCTCCGGAAGCAGAGCGGAGCGCTGTGTTTCCCTGTAAGTCTCGGTCAGGATATCCTGACCTTCACCTGCTCTCTAA
 CGGACGGGTCATCAGCGCTCCGGAAGCAGAGCGGAGCGCTGTGTTTCCCTGTAAGTCTCGGTCAGGATATCCTGACCTTCACCTGCTCTCTAA
 II CGGACGGGTCATCAGCGCTCCGGAAGCAGAGCGGAGCGCTGTGTTTCCCTGTAAGTCTCGGTCAGGATATCCTGACCTTCACCTGCTCTCTAA

A10B = scFv of anti-R-IgG antibody (Pharmacia)



scFv structure:



First Experiment:

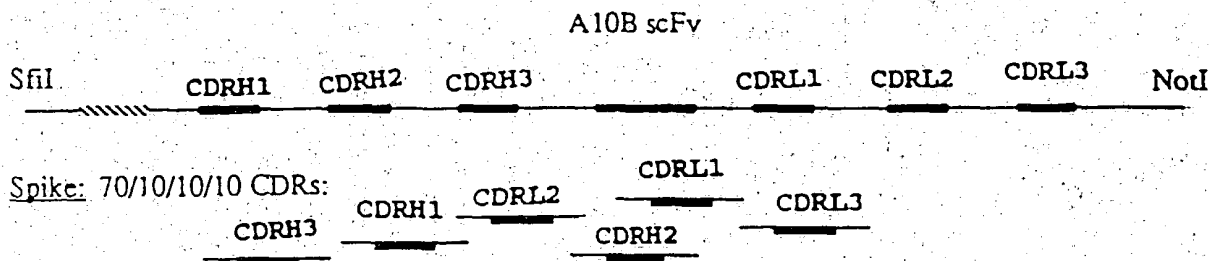


FIG. 6

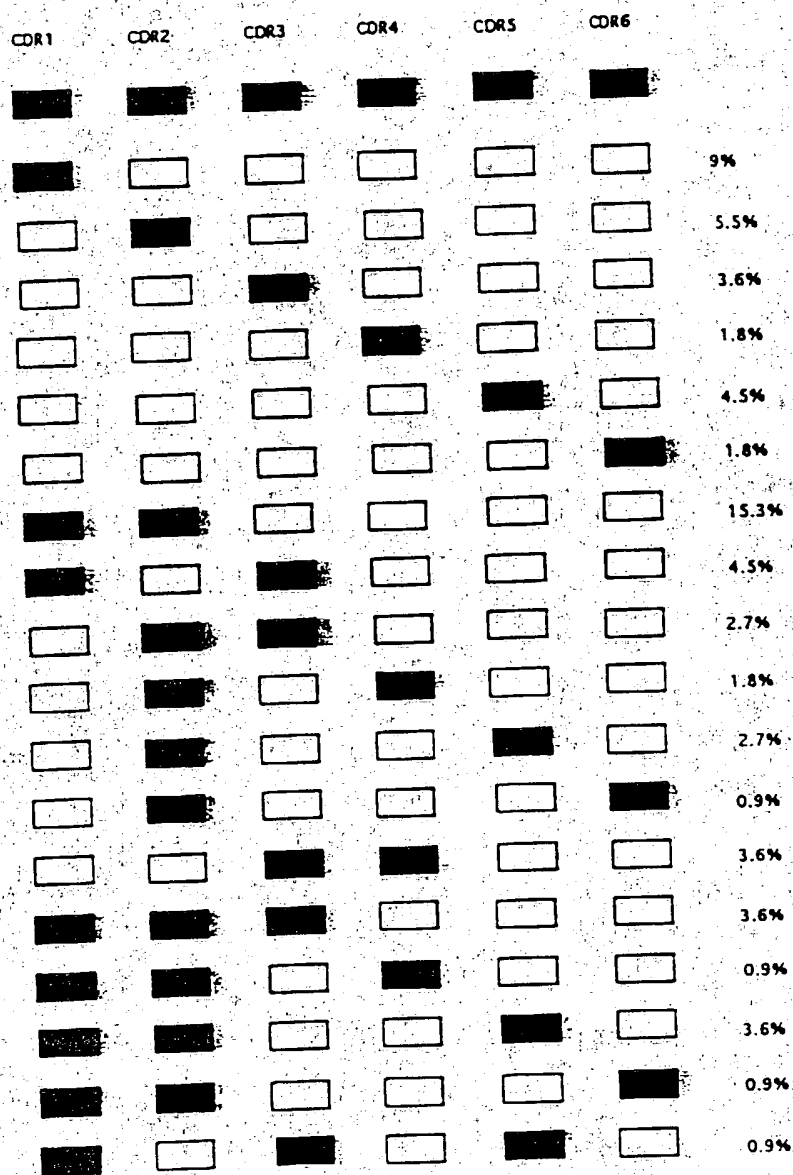


FIG. 7

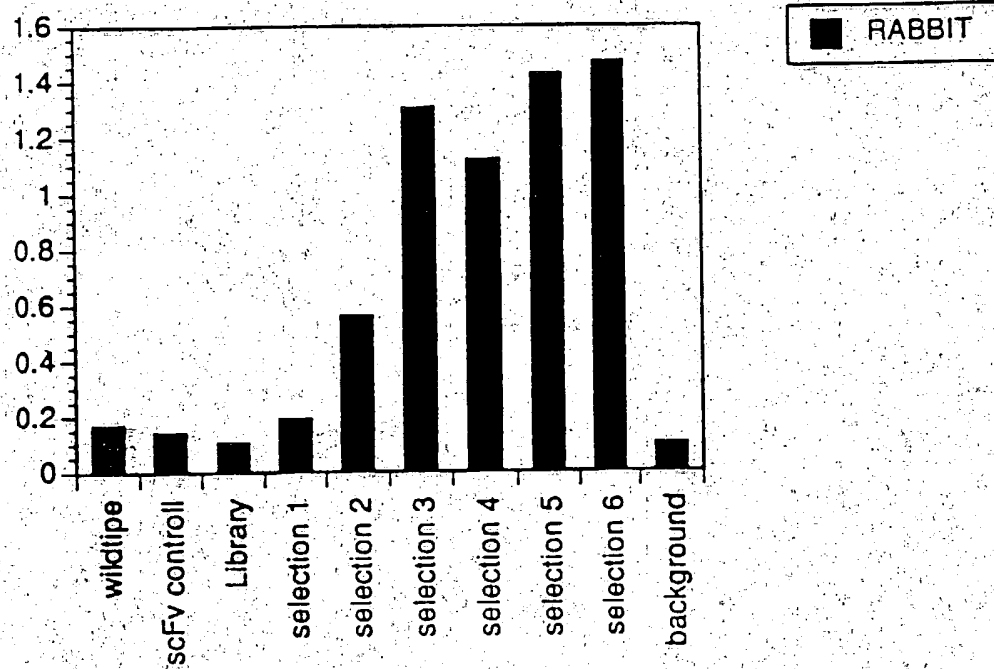
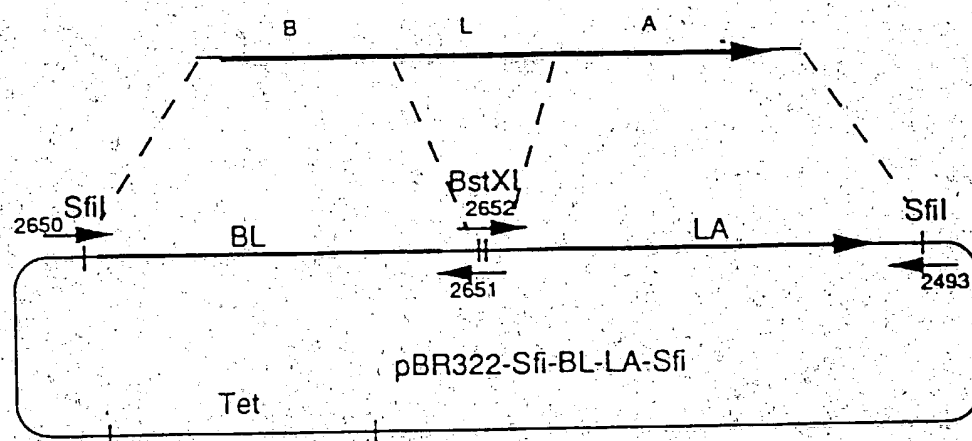


FIG. 8

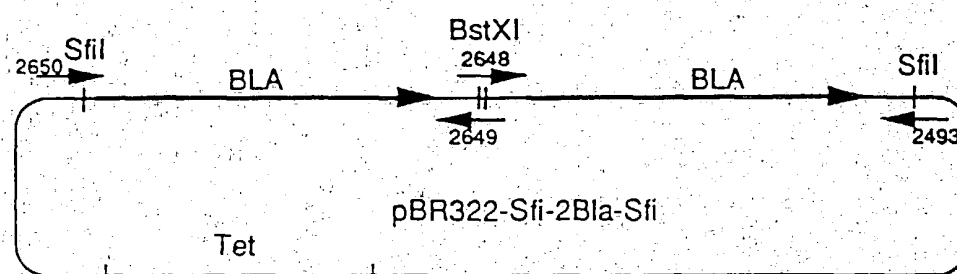
In vivo recombination via direct repeats - Intraplasmidic



Cell	Tet colonies	Amp colonies	Colony PCR
TG-1	131	21	3/3 at 1 kb
JC8679	123	31	4/4 at 1 kb
vector control	51	0	

FIGURE 9

In vivo recombination via direct repeats - Intraplasmidic

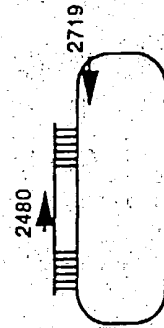


Cell	Tet colonies	Amp colonies	Colony PCR
TG-1	28	54	7/7 at 1 kb
JC8679	149	117	3/3 at 1 kb
vector control	51	0	

FIGURE 10

Homologous Recombination by Fragment Electroporation

Approach	Amp colonies	Amp Tet colonies	% homologous recombination	Comment
1- cut vector 1 insert JC8679	4,000	1,500	100% (N=14)	Efficient insertion by homologous recombination with co-electroporated vector
2- cut vector 2 inserts JC8679	2,000	16	100% (N=2)	100x less efficient than 1 fragment
3- uncut vector 1 insert JC8679	16	0		Homologous insertion depends on free ends.
4- no vector 1 insert JC8679::pUCSfi-Sfi	5,000	10,000	70% (N=7)	If vector is in cells already, high efficiency occurs even though vector is uncut
5- no vector 1 insert JC8679	2,000	0		- control: non-homologous insertion into chromosome
6- cut vector no insert JC8679	N.D.	0		- control: No amp background



Homologous recombination colony PCR:

FIGURE 11

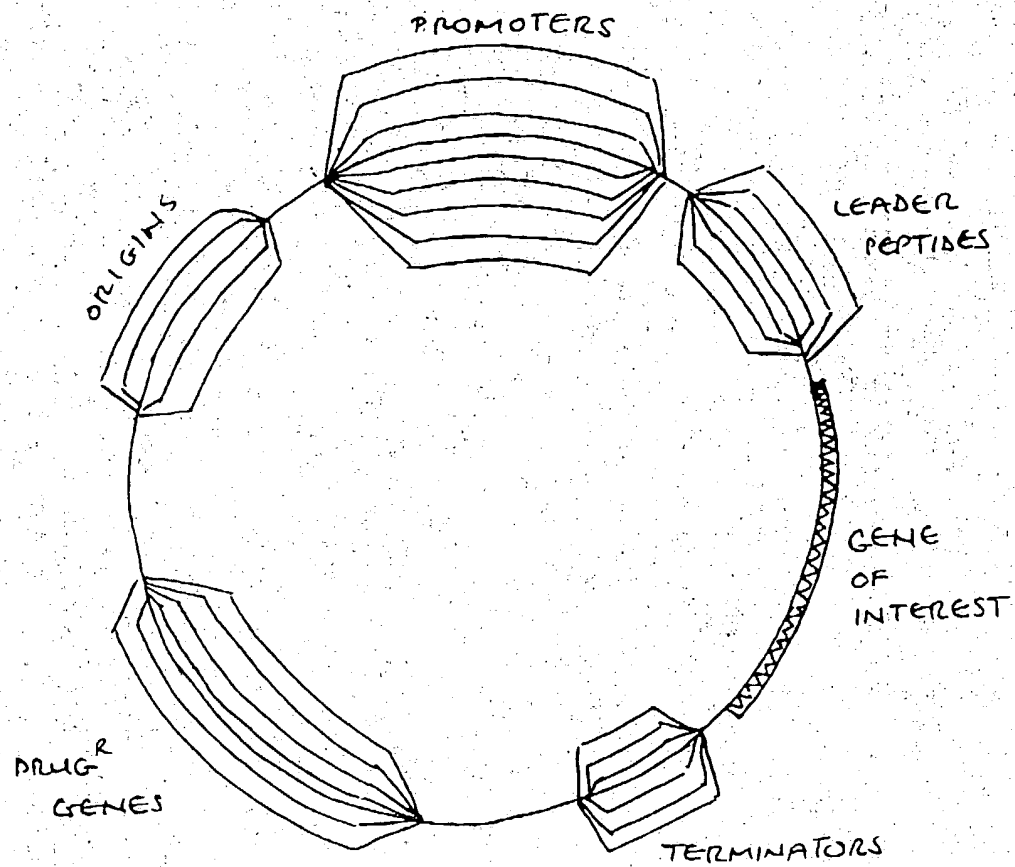
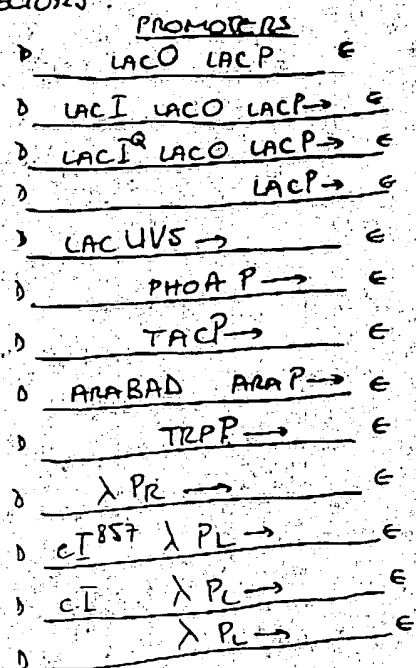
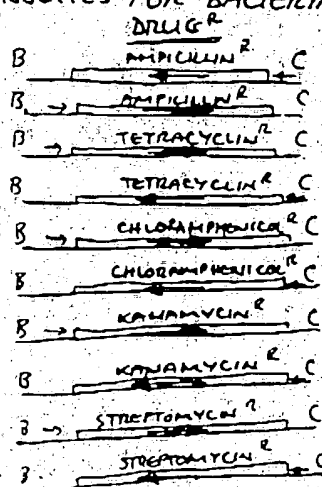
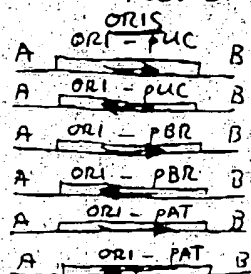
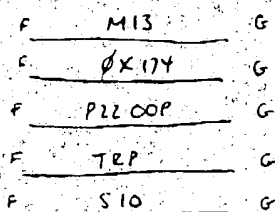


FIGURE 12

EXAMPLES OF CASSETTES FOR BACTERIAL VECTORS :



TERMINATORS



SS DNA ORI

M13

SIGNAL PEPTIDES

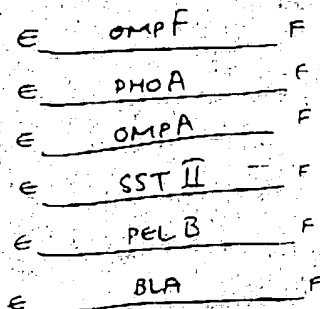


FIGURE 13